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INSTRUCTIONS FOR PREPARING AND SHIPPING PATHOLOGICAL SPECIMENS FOR DIAGNOSIS.[a]

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While laboratories for research in veterinary medicine are still but thinly scattered over our country, they are nevertheless sufficiently numerous to be within reach of the majority of sections. This, together with the present elaborate technique and scientific methods of diagnosing various obscure diseases of our domestic animals, makes it wise for veterinary inspectors and practitioners to acquaint themselves with the methods of obtaining and preparing tissues to be sent to the laboratory for pathological and bacteriological studies. The great assistance which the laboratory should be able to render to veterinarians and others in various disease conditions is in many cases reduced to a minimum by carelessness or indifference on their part in preparing the specimens before shipment. Where the proper measures are not taken, the specimens are often irretrievably spoiled for pathological work by the time they reach the laboratory, even though they are in a fresh condition when taken from the animal. In other cases, where a micro-organism is to be isolated to establish a diagnosis, it has become so badly contaminated by the abundant growth of saprophytic organisms that it is either never isolated or the time required is so great that the report of the laboratory is too long in being received to be of any material benefit to the interested person.

In many cases the mode of preparation of tissues must vary somewhat, depending upon whether they are to be used for pathological or bacteriological work. A dead body commences to undergo decomposition as soon as rigor mortis passes off, and in the case of the internal organs almost immediately on their exposure to the air. All solutions which will rapidly kill and properly fix the individual cells of a tissue are antiseptic and will render hopeless the procuring of any cultures. On the other hand, tissues not placed in such solu-

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tions will undergo more or less putrefactive changes during transit, which may destroy the delicate arrangement and staining properties of the cells so essential to proper pathological study. It must therefore be first considered which of the two is the more important in rendering the desired information, and, if possible, a method should be secured which will answer for both, at least in a certain percentage of cases.

In all forms of infectious diseases the isolation of the specific organism is the crucial test in establishing the diagnosis, and therefore the bacteriological examination is the more important. On the other hand, in cases of tumors, cirrhosis of the liver, nephritis, etc., a bacteriological examination gives no positive information and the entire diagnosis rests on the microscopic examination of sections. In many instances, however, these two branches of medical science overlap each other and both are necessary in establishing the nature of the diseased condition. In such cases two sets of tissues have to be prepared during warm seasons or where the time for transit requires thirty-six hours or longer.

TISSUES TO BE FORWARDED.

The selection of the organs or parts of organs to be forwarded requires careful consideration. In cases where the infection is general, as in many infectious diseases, all the viscera as well as the blood will contain the causative organisms. In such cases organs for bacteriological examination should be taken which are least liable to be contaminated post-mortem, as examples of which may be mentioned the heart, the spleen, and the lymphatic glands. The liver and the pancreas, on account of their direct communication with the intestinal tract through their excretory ducts, are more liable to infection with saprophytic organisms than the above, which have no communication with the exterior. It must be remembered, however, that in any condition where the lesion appears to be local the importance of sending the affected viscera, as the liver or even the intestine, which always contains many varieties of bacteria, greatly outweighs the increased danger of contamination with other organisms. Where any doubt exists as to whether the condition is local or general, the spleen or the heart should be forwarded in conjunction with the seemingly localized lesions.

GENERAL DIRECTIONS.

In case a number of animals are showing symptoms of an unknown or obscure disease, it is better to obtain one which has recently died, or to kill one of the worst affected cases for post-mortem examination, and also to obtain fresh material to send to the laboratory for diagno-

sis. Where the disease is devastating the smaller animals, as poultry, or even sheep and swine, a positive diagnosis is more likely to be obtained by sending to the laboratory by express one of the animals showing well-advanced symptoms.

In making the post-mortem examination in the field, if the operator has any idea that laboratory assistance will be necessary in rendering a diagnosis he should keep the fact constantly in mind during the procedure. Immediately upon opening the large body cavities the parts to be forwarded should be at once removed. As a general routine, the spleen and the heart are the organs which should be forwarded for a bacteriological examination, and they should be removed with the greatest caution and handled as little as possible. The heart should have all the large vessels ligated with string which has been immersed in a solution of bichlorid of mercury, 1 to 1,000, or in some other antiseptic solution. The knife and forceps used in removing the viscera should be either flamed over an alcohol lamp or over the flame of a match, or washed in an antiseptic solution, as 5 per cent carbolic acid. After removal the viscera should be laid separately on cheese cloth which has been saturated and thoroughly wrung out of bichlorid of mercury solution, 1 to 1,000, and wrapped up with several layers of the material. They should then be wrapped in paraffined or oiled paper and placed in a box for shipment. In the case of small animals the entire organs should be forwarded, as sections which necessitate the exposure of the parenchyma increase the liability to invasion with extraneous bacteria. In the case of large animals a portion of the spleen may be cut off, especial care being taken that the knife is sterile. The heart, however, must always be left intact. In addition to these organs, such other parts as show especially marked or peculiar lesions should also be forwarded, each piece being wrapped separately in the bichlorid gauze.

When intestines are to be forwarded the section should be removed and the excess of alimentary contents washed away by dipping in clear water. If the bowel is empty, it is better to tie both ends before removing and wrap in the gauze immediately, without washing. If during removal the tissues become covered with blood, it should be wiped off with gauze wrung out of bichlorid solution and not washed off in water. Removal of any foreign bodies, as dirt, straw, etc., should never be necessary, as such contamination should be scrupulously avoided.

Covering the tissues with a thick layer of powdered boric acid and wrapping in dry gauze is also practiced considerably and is equally valuable as the damp gauze, but has the disadvantage of being harder to remove at the laboratory. Juicy or friable tissues had better be placed in sterile or thoroughly clean glass containers

without wrapping. Under ordinary conditions when the tissues are taken immediately after death and the laboratory is near by, the above mode of preparation preserves the tissues sufficiently well for pathological work. During exceptionally warm weather, however, or when friable parts are to be sectioned, and especially when tumors are to be forwarded, other methods have to be used in the preparation. In these cases small plugs of tissue one-half to 1 inch square are all that are required. These should be cut, whenever possible, so as to include a part of the normal tissue with the diseased areas. The knife should be reasonably sharp to prevent crushing the connective tissue framework and destroying the cell arrangement. Strict antiseptic precautions are not necessary in securing such pieces of tissue, as they are to be immediately placed in antiseptic solution and no cultures are desired. Probably the most easily obtained and efficient fluid to forward such tissues in is a 4 per cent solution of formaldehyde gas in water (10 per cent solution of formalin), the quantity of which should be from 10 to 20 times the volume of the tissues to be placed in it. Alcohol may also be recommended in the absence of the above fluid, but it should be at least 80 per cent strength; 95 per cent is preferable. Orth's fluid, consisting of—

	Parts.
Bichromate of potassium	2
Sodium sulphate	1
Water	100
Formaldehyde, 40 per cent	10

is better than either of the above, but the potassium bichromate is not always obtainable, and the formalin alone is sufficient. If it is to be used, the formalin should not be added until just prior to shipping, as it leads to precipitation in a few days.

SPECIAL METHODS OF PREPARING TISSUES.

There are some important diseases in which the laboratory worker would be unable to render a diagnosis from the previously mentioned tissues. As examples may be mentioned rabies, cerebro-spinal meningitis, tetanus, etc. In the latter two diseases the history and symptoms are usually sufficient to make a diagnosis, and the tissues frequently give but little information. Some special directions may, however, be given in regard to rabies, anthrax, tuberculosis, and glanders.

RABIES.

Rabies is a disease in which the laboratory is able to give very material aid. In cases where persons have been bitten by a suspected rabid animal a laboratory diagnosis is often demanded even though competent and experienced veterinarians have pronounced the case

rabies from a careful observation of the symptoms and course of the disease.

When a dog or other animal has died of suspected rabies and a positive diagnosis is desired, the carcass should be autopsied to exclude other causes of death. Particular attention should be paid to the stomach to ascertain the presence of foreign bodies and any inflammatory condition of the gastric mucous membrane, both of which are indicative but not at all conclusive signs of rabies. The head with the skin intact should then be removed by cutting through the middle of the cervical vertebræ; it should be wrapped in dry cheese cloth or other material and forwarded by express. During very warm weather, after wrapping the head, it should be placed in a tin receptacle and packed in a wooden box containing chopped ice. By removing the head at the middle of the cervical vertebræ the plexiform ganglia are left intact and upon arrival at the laboratory they can be removed and examined microscopically for the lesions described by Van Gehuchten and Nelis and a diagnosis made within twenty-four hours. This is not practicable when several days are required for the head to reach the laboratory, as the brain undergoes softening, becomes invaded with bacteria, and the experimental rabbits inoculated are liable to die from septicemia. Putrefactive changes are also liable to occur in the ganglia and thus render the conclusions from their examination indefinite.

In such cases the brain, including the medulla oblongata, should be removed as carefully as possible in one piece, immersed in two to three times its volume of pure neutral glycerin, and sent in this manner. In large animals one cerebral hemisphere and the medulla are sufficient. In some cases even with this method the Negri bodies can be demonstrated in the large nerve cells of the hippocampus major and thus a diagnosis made in a few hours without waiting for the rabbits to develop the disease, which requires from two to three weeks.

It must be remembered, however, that to get the best results with the rapid methods of diagnosis it is essential that the animal be allowed to die naturally from the disease, as when killed in the early stages the changes in the central nervous system have frequently not developed sufficiently to be recognized.

ANTHRAX.

In cases of anthrax a post-mortem examination should be made with great care or not at all. The *Bacillus anthracis* only forms spores in the presence of oxygen, and therefore so long as the carcass is left intact spore formation does not occur. From the fact that the spores are so resistant and infection of the premises is such a

serious matter, it is advised by some authorities not to make a post-mortem examination. Instead remove one ear from the carcass, wrap it in gauze wrung out of a 1 to 1,000 solution of bichlorid of mercury and forward this to the laboratory, relying entirely upon the bacteriological examination to establish the diagnosis. This may also be accomplished by allowing two or three drops of blood from the ear to drop at different points on a piece of ordinary writing paper. After it has thoroughly dried it may be folded inside a second piece of paper and forwarded in a mailing case.

TUBERCULOSIS.

In tuberculosis, while blood infection does occur, the organisms can rarely be demonstrated in the blood, and therefore unless the heart or spleen show lesions of the disease they are of no value in diagnosing the condition. The peculiar staining properties of the tubercle bacillus render it easy to recognize, even when very badly mixed with other organisms. It must be remembered, however, that in old tuberculosis lesions it is very difficult, and often impossible, to demonstrate the tubercle bacillus. Therefore in sending tuberculous tissues care should be taken to get the young lesions, or those with areas of inflammation about them which show that the process is actively going on and thereby assures the presence of the tubercle bacillus provided the disease is tuberculosis. Such tissues should be wrapped in the bichlorid gauze, as previously described.

GLANDERS.

In forwarding material from suspected cases of glanders for the guinea-pig test of Strauss, the nasal discharge, or the oily serum from a farcy bud, should be selected. It is best obtained on a cotton swab prepared in the following manner: A piece of absorbent cotton is wrapped about the end of a piece of thick wire or portion of an umbrella rib. This should then be placed inside of a glass tube and the latter plugged with a cotton plug, the end of the wire being allowed to project beyond the tube. The plugged tube containing the swab should then be sterilized by dry air or steam. When this sterilization is impossible, the tube and wire should first be boiled, and with thoroughly cleaned hands the swab and plug can be made from sterilized absorbent cotton as purchased from pharmacists.

In collecting the material an assistant should hold the animal's head. The veterinarian holds the tube and plug in the left hand, while with the the right hand he withdraws the swab from the tube by grasping the end of the wire; he then passes it up into the nostril, turns it once or twice to collect the discharge, and replaces it in the tube, carefully inserting the plug. It should be taken to the lab-

oratory in person, if this is possible, as any considerable delay would render the material useless.

Where the diagnosis of glanders by the serum agglutination reaction of McFadyean is desired, it is essential that the blood be drawn in a sterile condition. For this purpose 25 c. c. of blood is sufficient, and it is best obtained from the jugular vein. The site of operation should have the hair clipped away or shaved and thoroughly washed with soap and water followed by some antiseptic solution, as 5 per cent carbolic acid or 1 to 1,000 bichlorid of mercury. The trocar used in making the puncture, as well as the tube or bottle with a rubber stopper in which the blood is to be collected, must have boiled for fifteen minutes just prior to being used. The results from this test, however, are in some cases indefinite, and when the animals are showing symptoms of the disease Strauss's test is more reliable. Its chief value lies in detecting incipient glanders in a stable where acute cases have recently developed and the results of the mallein tests are indefinite.

CLINICAL LABORATORY WORK.

This is but rarely taken advantage of among veterinary practitioners. Several good reasons may be given for this, among which may be mentioned (1) the fact that in many cases the economic value of the patient is not sufficient to justify the procedure; (2) the laboratory making the examinations must be close at hand; and (3) the value of such work is not generally recognized by the profession.

Blood counts are rarely made, while hemoglobin estimations, though more frequent, are still not in general use. Urine analysis is also infrequently resorted to for diagnostic purposes, although it is probable that if this were to become a routine practice many cases of kidney disease would be diagnosed which at present are never recognized.

In sending urine to the laboratory for analysis it is preferable to have a portion of a 24-hour sample forwarded, but this is practically impossible, and therefore a sample has to be secured whenever opportunity offers. From 150 to 200 c. c. should be placed in a previously boiled glass container, and, as many bacteria frequently present in urine have a disintegrating effect on casts and other cellular elements in the urine, the specimen should reach the laboratory in the shortest possible time.

MILK.

In securing milk for a bacteriological examination the udder and teats should first be thoroughly washed off with soap and warm water, followed by some antiseptic solution, after which the excess of fluid should be wiped off with a clean towel. The first two or three streams of milk should be discarded, after which it should be

milked directly into a previously boiled glass bottle. For ordinary examination 50 c. c. is sufficient. It must, however, be forwarded expeditiously, as milk is an ideal culture media for germs, and they multiply very rapidly in it.

SKIN DISEASES.

In skin diseases a differentiation between parasitic and nonparasitic diseases, as well as the nature of the parasite, if present, can often be made by microscopic examination of the scrapings. Thus considerable assistance may be obtained in rendering a prognosis as well as in the methods of treatment. Such scrapings should be fairly deep, at least into the true skin. They may be merely wrapped in paper and mailed in an ordinary envelope or forwarded in small homeopathic glass vials. They must never be taken, however, until at least twenty-four hours after applying any treatment to the parts, as this will very likely destroy all the parasites in the superficial layers of the skin and render their detection difficult or impossible.

Ticks which may be suspected of being the *Boophilus annulatus*, the carrier of the causative agent of Texas fever, or any other external parasites, may be forwarded for identification by placing a few specimens, both male and female, in a small glass bottle, lightly corked with cotton, or any other convenient container, and mailing it to the laboratory in a mailing case.

MUSEUM SPECIMENS.

When especially well-developed or unusual lesions are found in any animal the laboratory is often desirous of preserving the entire affected organ or part as a museum specimen. When such a procedure is contemplated it is necessary, in order to obtain the best results, that the parts be placed in the preserving fluid before they have undergone any post-mortem decomposition and without their surfaces becoming dry to any considerable degree.

The method of Kaiserling is undoubtedly the best method of preserving tissues so as to retain their natural color. This method consists in placing the tissues in a fixing solution consisting of—

Potassium nitrate	grams	15
Potassium acetate	grams	30
Formaldehyde	c. c.	200
Water	c. c.	1,000

After having been left in this solution a variable length of time, depending on their size and consistency, they are removed, passed through increasing strengths of alcohol to restore the color, and finally preserved in—

Potassium acetate	grams	200
Glycerin	c. c.	400
Water	c. c.	2,000

It is only necessary to prepare the first solution in the field and forward the specimen in this to the laboratory. If the potassium salts can not be procured, the tissues may be fairly well preserved until they reach the laboratory by merely using the formaldehyde and water. Alcohol is also a very good preservative and more economical than the preceding, but it has the disadvantage of not maintaining the natural color of the specimen for any length of time. When preservation of color is not essential alcohol is probably the best simple substitute for Kaiserling's method.

LABELING AND INFORMATION TO ACCOMPANY SPECIMENS.

After wrapping each piece of tissue separately in gauze it is sufficient to have one container for all the specimens from one animal, but in no case should specimens from different animals be put in the same container, as they are often hard to differentiate at the laboratory. The separate containers, however, may all be packed in one box. Each container should be labeled with the name of the various tissues which it contains as well as the species of animals from which they were taken.

It is also very important for all packages to have written or painted on them the name and address of the sender. Within a period of five months three specimens were received at this laboratory unaccompanied by letters and with nothing on the packages except the postmark to indicate their origin. One was a tuberculous spleen, the container of which was postmarked Chicago, but although a letter diagnosing the case was forwarded to the inspector in charge of meat inspection at that station, the sender was not found. Another specimen, a case of demodectic mange affecting the skin of a hog, was received under similar conditions, while the third consisted of tuberculous lymph glands from a hog. These were all quickly and easily diagnosed, but the senders remain in ignorance of the results because they failed to write concerning the specimens and failed to mention their origin on the packages.

Specimens should always be accompanied by a letter giving full information regarding the particular case from which they were taken, as to age, history, symptoms, post-mortem findings, etc. In addition to this, where a number of animals are affected, complete details of the outbreak should be given, together with any peculiar symptoms shown by one or more of the cases; whether there had been any similar outbreaks; if animals on near-by farms were showing the same trouble; the number of animals that had died, and such other information as is liable to be of assistance to the laboratory worker in drawing conclusions from the results obtained.

Below will be found the postal regulations relative to the mailing of diseased tissues. Where the packages are too large for mailing,

or if the use of the mails is undesirable for other reasons, the specimens should be forwarded by express to the Chief of Bureau of Animal Industry, Pathological Division, Washington, D. C., with the name and residence of the shipper plainly indicated. Mail packages should be similarly marked.

EXTRACTS FROM POSTAL LAWS, REGULATIONS, AND CONVENTIONS, 1902.

SEC. 495. Specimens of diseased tissues may be admitted to the mail for transmission to United States, State, or municipal laboratories only when inclosed in mailing packages constructed in accordance with this regulation.

2. Liquid cultures, or cultures of micro-organisms in media that are fluid at the ordinary temperature (below 45° C. or 113° F.) are unmailable. Such specimens may be sent in media that remain solid at ordinary temperatures.

3. Upon the outside of every package of diseased tissues admitted to the mails shall be written or printed the words "Specimen for Bacteriological Examination. This package to be treated as letter mail."

4. Packages used for conveying through the mails pathological specimens for bacteriological examination for diagnosis in cases of suspected diphtheria, tuberculosis, and other communicable diseases shall be constructed and prepared as follows:

a. The receptacle for moist specimens of diseased tissues shall be a strong glass vial or test tube having a capacity not greater than 2 drams. The vial shall be covered and made water-tight by the use of a metal screw cap and a rubber or felt washer which has been immersed in melted paraffin, or, if a test tube be used, it shall be covered with a tightly fitting rubber cap.

b. The vial or test tube shall be placed inverted in a circular tin box, which shall be made of I. C. bright tin plate, and have flush or countersunk bottom and soldered joints and not be smaller than $1\frac{1}{8}$ inches in diameter and 3 inches long, nor larger than $2\frac{1}{4}$ inches in diameter and $5\frac{1}{2}$ inches long. This box shall be closed by a metal screw cover and a rubber or felt washer, or tightly fitting metal sliding cover, and shall be so packed with absorbent cotton, closely laid, that the glass or test tube contained therein shall be evenly surrounded on all sides by cotton.

c. The tin box shall be placed inverted inside of a larger tin box similar to the one already described, which should snugly receive the specimen box. Upon the inside of the sides and bottom of this outer box there shall be a lining of compressed paper not less than three-sixteenths of an inch in thickness. This outer tin box shall be closed by a metal screw cap and a rubber or felt washer. This outside box may also consist of hardwood in the form of a block with a cylindrical hole bored in one end and extending to within not less than 1 inch of the opposite end; the open end to be closed with a wooden or metal screw cap with a rubber or felt washer. Or the outside box may be a cylindrical wooden box having a screw cap and washer. The thickness of the sustaining part of the wooden tube must be not less than one-quarter of an inch and be lined same as the tin box.

d. The receptacle for dry specimens of diseased tissues shall be a glass test tube 3 inches in length and one-half inch in diameter. This test tube shall be inclosed in a circular tin box similar to those already described, but measuring $2\frac{1}{4}$ inches in diameter and $5\frac{1}{2}$ inches in length, and be lined upon its sides and bottom with compressed paper not less than one-quarter of an inch in thickness. The test tube shall be closely packed in cotton, and the box shall be closed by a metal screw cap and a rubber or felt washer.

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